

AWARD NUMBER: W81XWH-14-2-0153

TITLE: Decreasing Skin Graft Contraction through Topical Wound Bed Preparation with Anti-Inflammatory Agents

PRINCIPAL INVESTIGATOR: Rodney Chan, MD

CONTRACTING ORGANIZATION: The Geneva Foundation  
Tacoma, WA 98402

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# REPORT DOCUMENTATION PAGE

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> This proposal aims to identify topically applied anti-inflammatory drugs that will reduce recipient site inflammation and skin graft contraction.					
<b>15. SUBJECT TERMS</b> Graft contraction, anti-inflammatory, hypertrophic scaring					
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## 1. INTRODUCTION:

We hypothesize that the elevated and prolonged inflammatory state of the recipient wound bed is a causative factor in the development of skin graft contraction. Using a porcine model of skin graft contraction, we will screen for anti-inflammatory agents (dose, schedule of administration, drug class) that reduce inflammatory cytokines in the recipient wound bed during 6 days post-wounding. We will then validate the effectiveness of the anti-inflammatory agent, dose and schedule to reduce contraction of the grafted split-thickness skin by allowing the experimental animal to survive for a longer period of time. Specifically, the aims of the proposal are to develop treatments that modulate inflammation and decrease skin graft contraction. We will achieve this by (1) identifying a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers and (2) validate the schedule and dose of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction.

## 2. KEYWORDS:

Skin graft contraction; burn; prolonged inflammation; anti-inflammatory; wound bed.

## 3. ACCOMPLISHMENTS:

*What were the major goals of the project?*

As stated in the approved SOW, the Specific Aims of the project is as follows:

### **Develop treatments that modulate inflammation and decrease skin graft contraction**

A.1.2.1 Identify a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers.

A.1.2.2 Validate the schedule and dose of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction.

The initially proposed statement of work is illustrated below with the number of animals used relative to the number of animals proposed.

### Project Milestones:

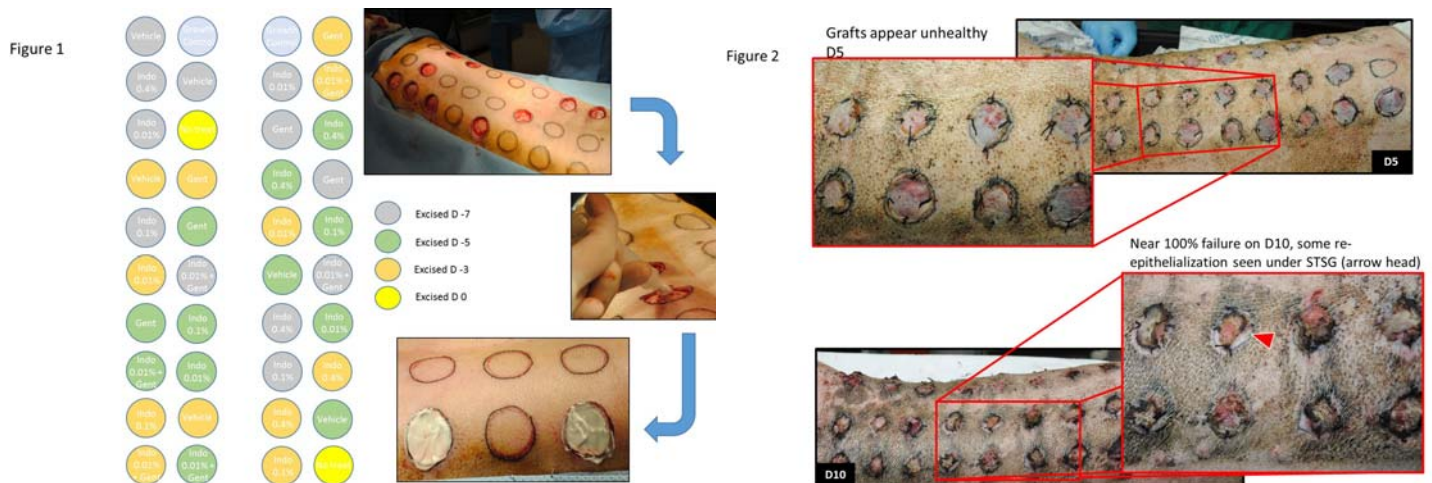
MILESTONES FOR THE RESEARCH PROGRAM		Year 1	Year 2
<b>Specific Aim:</b>	<b>Develop treatments that modulate inflammation and decrease skin graft contraction</b>		
<b>A.1.2.1</b>	- Identify a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers.	6* (Proposed) 2 (Used to date)	
<b>A.1.2.2</b>	- Validate the schedule and dose of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction.		3 (Proposed) 1 (Used to date)

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\*number of animals, included one replacement animal per experiment

### What was accomplished under these goals?

In the first goal (A.1.2.1), the screening animal models using the Yorkshire pig for burn and excisional wounds protocol was approved both by ISR IACUC and MRMC ACURO. In the first experimental animal, 40 wounds were created on the back of the pigs randomized to be excised at different times so as to result in different periods of open wound. The treatments included vehicle alone, treatment with different concentration of indomethacin containing antibiotics and antibiotics alone (See Figure 1). All the wounds were then grafted on the same date but for unknown reasons resulted in near complete graft failure (See Figure 2). For this reason, we postulated that the failure may be secondary to indomethacin toxicity, infection or technical failure.



In the next experimental animal, in order to rule out the possibility for indomethacin toxicity, the groups were limited to only vehicle. This still resulted in an unacceptably high level of graft failure (See Figure 3). At this time, the next experiment is to perform grafting without an antecedent period of wound bed preparation in order to rule out the possibility of infection.

Figure 3 Vehicle Only

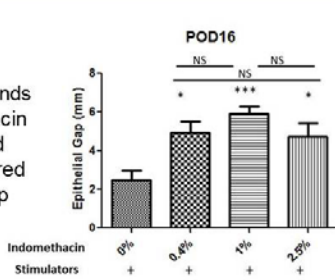


In order to concurrently assess the use of anti-inflammatory strategies in a separate animal model as an addition screening experiments, preliminary studies using rabbit excisional wounds treated with low dose indomethacin (from 0.4% to 2%) were studied. This resulted in delayed wound healing (top left graph) but also a decreased scar elevation index (bottom left graph). The 0.4% formulation resulted in only a modest delayed in wound

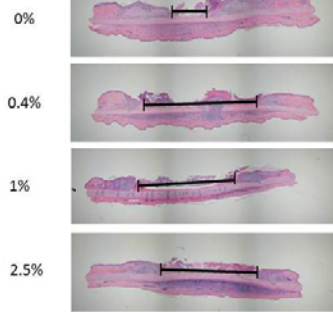
healing but significantly decreased scarring. Using the rabbit model as preliminary evidence, we were able to narrow down the therapeutic window for the concentration of anti-inflammatory indomethacin to be used for the porcine studies once the technical difficulties with graft loss is resolved.

**Treatment of acutely inflamed wounds with indomethacin delays wound closure**

Treatment of stimulated wounds with indomethacin retarded wound closure measured by epithelial gap



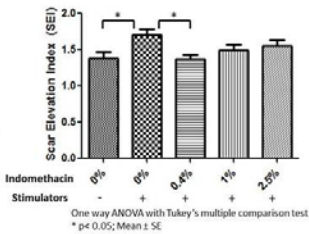
Indomethacin



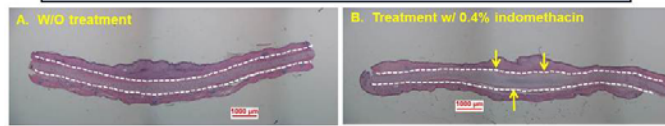
H&E staining of histological sections of wounds stimulated with combined heat-killed bacteria + dermal necrotic tissue and treated with different doses of indomethacin. Wounds were harvested and processed on POD16

**Low-dose Indomethacin (0.4%), but not higher doses, reduced scarring of acutely inflamed wounds**

Scarring is expressed as Scar Elevation Index (SEI). SEI is a ratio of total scar area to the estimated area of non-scarred dermis

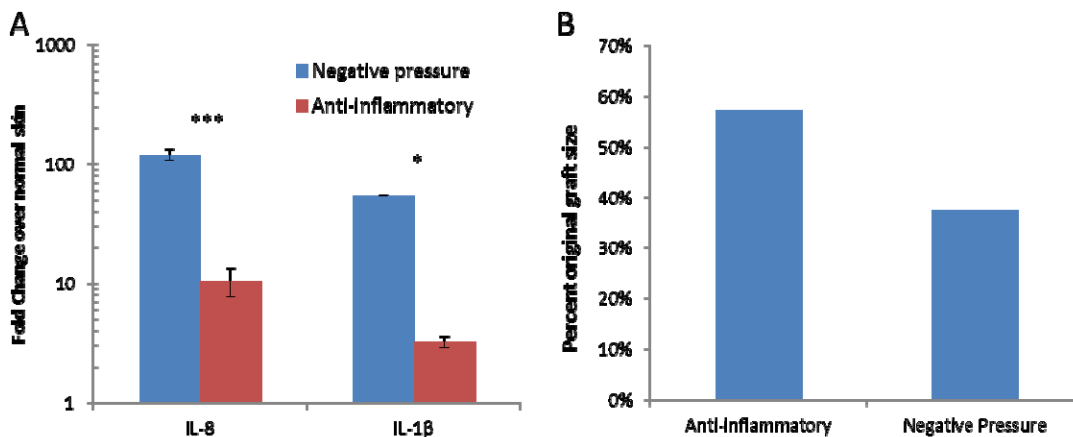


**Treatment of acutely inflamed wounds with Indomethacin causes "hypertrophy" of cartilage**



Cartilage is illustrated by the white dotted lines. Arrows depict an hypertrophic area.

In the second goal (A.1.2.2), in the approved large animal model of wound creation and wound bed modulation, experiments were performed to validate the model for experimental conditions found in the smaller wounds. At this point, we have done one experimental animal and in that animal, wounds dressed using a negative pressure dressing revealed greater inflammation than wound treated with an anti-inflammatory (See Figure below). Grafting did not occur and will take place after experimental conditions are better worked out from Aim 1.



A porcine model of excisional wounds was developed to study the effect of wound bed modulation on eventual graft contraction. (A) Anti-inflammatory treatment of the wound bed reduced expression of inflammatory cytokines compared to standard negative pressure dressing. (B) Reduction in inflammatory markers coincided with reduced contraction compared to standard negative pressure dressing. \* =  $p < 0.05$ ; \*\*\* =  $p < 0.001$ . This preliminary data points to a positive influence of reducing inflammation on graft contraction. The results of this study may have imminent clinical relevance to decrease skin graft contraction and late contractures, thus reducing the need for multiple late revisional operations.

*What opportunities for training and professional development has the project provided?*

The proposal presents multiple opportunities for training and professional development.

Training- Courses: None

Training- One-on-One work with Mentor: Both the Post-doc and Research Technician has spent time with PI for one-on-one training with surgical techniques. Both the Post-doc and Research Technician has spent time with a dermatopathologist for one-on-one training for interpretation of histological findings.

Professional Development- Workshops/Seminars: The Study PI, Co-PI, Post-Doc and Research Technician has had opportunities to present their portion of the work in the burn scar mitigation group weekly research seminar and the wound healing group bi-monthly research seminar. The progress of this work has also been presented at the USAISR wide research meeting.

Professional Development- Group/Individual Study: None

*How were the results disseminated to communities of interest?*

The interim results of the study have been presented in the burn scar mitigation group weekly research seminar and the wound healing group bi-monthly research seminar. The progress of this work has also been presented at the USAISR wide research meeting.

*What do you plan to do during the next reporting period to accomplish the goals?*

In the next reporting period, we plan to resolve the issue that we are having with graft failure and then move on to examining the proper topical class of anti-inflammatory that will result in diminished inflammation. This will be translated in to the second aim where this class of anti-inflammatory may result in diminished skin graft contraction.

#### **4. IMPACT:**

*What was the impact on other disciplines?*

Dysregulated inflammation has been implicated in many diseases of the human body. The findings of this experiment is particularly relevant as they relate to dysregulated inflammation of skin diseases. While the proposal has direct implications to scarring after trauma and burns, additional skin related diseases with an exacerbated inflammatory response include eczema, psoriasis and other forms of dermatitis. The approach to investigating the class and dose of topical anti-inflammatory formulation that is useful in the attenuation of scarring may be readily use to study the late effects of these other inflammatory conditions.

*What was the impact on technology transfer?*

Nothing to Report.

*What was the impact on society beyond science and technology?*

Nothing to Report.

#### **5. CHANGES/PROBLEMS:**

*Changes in approach and reasons for change*

Nothing to Report.

*Actual or anticipated problems or delays and actions or plans to resolve them*

Several problems has arisen during the course of this year to delay the progress of the experiments.

- (1) During the third quarter, the ISR contract with the pig vendor had lapsed and we were not able to order any animals for two months. This problem was resolved after the contract was renewed.
- (2) We were limited by the number of pig cages in the ISR vivarium due to conflicting studies from other groups. For these reasons, we were unable to perform new pig surgery on several occasions which have led to delay in the progress of this proposal. We have been working on a new MOU with another adjacent facility (TSRL- Tri-Service Research Laboratory) to perform portions of the porcine studies so that this problem may be mitigated in the future.
- (3) In performing the screening experiments in Aim 1, we encountered an unusually high rate of graft loss (see above) and hypothesized that it may be secondary to indomethacin toxicity, infection or technical problems. This required additional animals to elucidate these possibilities and resulted in delay of progress.
- (4) While personnel recruitment has not been an issue for the first year of this award, it is a potential source of delay in the second year. New government security clearance regulations require that personnel has to be in the country for 3 years before a security clearance can be performed, this will limit the pool of potential candidates to fulfill research needs.

*Changes that had a significant impact on expenditures*

Less cost has been incurred at this time in the hiring of a research technician to support this project. This is because a technician supported on intramural funds (Mr. Jesse Wu) has been assigned to this research project but this finished the end of the third quarter. We are in the process of recruiting a technical personnel for the remainder of the study.

*Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents*

Nothing to Report.

**6. PRODUCTS:**

*Publications, conference papers, and presentations*

Nothing to Report.

*Journal publications.*

No publication has been submitted yet based on the specific aims of this proposal. However, preliminary studies guiding experimental conditions of Aim 1 has been formulated into the follow manuscript.

Qian LW, Fourcaudot A, Kazuyoshi Y, You T, Chan RK, Leung KP. Exacerbated and prolonged inflammation impairs wound healing and increases scarring. Wound Repair and Regeneration. Accepted and in Press.  
Acknowledgement of Federal Support – Yes.

*Books or other non-periodical, one-time publications.*

Nothing to Report.



*Other publications, conference papers, and presentations.*

Nothing to Report.

*Website(s) or other Internet site(s)*

Nothing to Report.

*Technologies or techniques*

Nothing to Report.

*Inventions, patent applications, and/or licenses*

Nothing to Report.

*Other Products*

Nothing to Report.

## **7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

*What individuals have worked on the project?*

Name: Rodney Chan, MD  
Project Role: PI  
Nearest person month worked: 6  
Contribution to Project: Dr. Chan is the PI of the award

Name: Kai Leung, PhD  
Project Role: Co-PI  
Nearest person month worked: 3  
Contribution to Project: Dr. Leung is the co-PI of the award

Name: Llyod Rose, PhD  
Project Role: Post-Doctoral Fellow  
Nearest person month worked: 12  
Contribution to Project: Dr. Rose is the Post-doctoral fellow who has supported this project.

Name: Jesse Wu, MS  
Project Role: Research Technician  
Nearest person month worked: 6  
Contribution to Project: Mr. Wu is the Research technician who has supported this project.

*Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?*

Nothing to Report.

*What other organizations were involved as partners?*

*Provide the following information for each partnership:*

Organization Name: United States Institute of Surgical Research

Location of Organization: 3698 Chambers Pass  
JBSA Fort Sam Houston, TX 78234

Partner's contribution to the project

- Facilities – Project staff use the partner's facilities for project activities
- Collaboration – Partner's staff work with project staff on the project
- In-kind support – Partner makes software, computers, equipment, etc. available to project staff

## **8. SPECIAL REPORTING REQUIREMENTS**

### ***QUAD CHARTS:***

See Attached QUAD Chart.

## **9. APPENDICES:**

# Decreasing skin graft contraction through topical wound bed preparation with anti-inflammatory agents

W81XWH-14-2-0153



PI: Rodney Chan MD/Kai Leung PhD

Org: USAISR/The Geneva Foundation

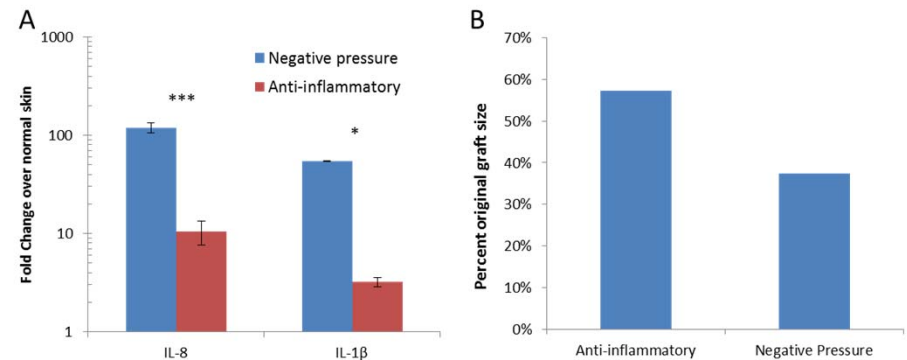
Award Amount: \$881,310

## Study/Product Aim(s)

1. Identify a dose and schedule of anti-inflammatory drug that most effectively blocks excessive inflammation of the recipient wound bed as defined by inflammatory markers.
2. Validate the dose and schedule of anti-inflammatory drug shown to reduce inflammation of the recipient wound bed to decrease skin graft contraction.

## Approach

A porcine model of excisional wound was developed to study wound inflammation and its effect on skin graft contraction. Wound bed modulation using anti-inflammatory treatments are first applied to a screening model and then validated on an experimental model with larger wounds to study skin graft contraction.



A porcine model of excisional wounds was developed to study the effect of wound bed modulation on eventual graft contraction. (A) Anti-inflammatory treatment of the wound bed reduced expression of inflammatory cytokines compared to standard negative pressure dressing. (B) Reduction in inflammatory markers coincided with reduced contraction compared to standard negative pressure dressing. \* =  $p < 0.05$ ; \*\*\* =  $p < 0.001$ . This preliminary data points to a positive influence of reducing inflammation on graft contraction. The results of this study may have imminent clinical relevance to decrease skin graft contraction and late contractures, thus reducing the need for multiple late revisional operations.

## Timeline and Cost

Activities	CY	14	15	16	17
Aim 1 (Identify drug/schedule/dose) using screening model			█	█	
Aim 2 (Identify drug/schedule/dose) using validation model				█	█
<b>Estimated Budget (\$K)</b>		<b>\$000</b>	<b>\$434</b>	<b>\$447</b>	<b>\$000</b>

## Goals/Milestones

**CY15 Goal** – Screening of anti-inflammatory therapies

- IRB Approval of both screening and validation porcine wound bed preparation model
- Establishment of Validation model to examine the effect of topical anti-inflammatory drugs
- Establishment of Screening model to examine the effect of topical anti-inflammatory drugs (in progress)
- Establish dose and schedule of anti-inflammatory drug best to decrease inflammatory markers (in progress)

**CY16 Goals** – Validation of anti-inflammatory therapies

- Establish dose and schedule of anti-inflammatory drug best to decrease inflammatory markers (continue from CY15)
- Validate the dose and schedule of anti-inflammatory drug best to decrease skin graft contraction

**Comments/Challenges/Issues/Concerns:** Encountered problems with an unacceptable rate of graft loss in the 3cm Wound in Aim 1. Experiments are ongoing to decipher whether the problem is secondary to infection, indomethacin toxicity or technical difficulties.

## Budget Expenditure to Date

Projected Expenditure: \$496,958

Actual Expenditure: \$ 110,000.23

Updated: 11 October 2015